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I, Janet Hope, BSc (Hons.), MIL., MITI., translator to Messrs. Taylor and Meyer of 20 Kingsmead Road, London, SW2 3JD, Great Britain, verify that I know well both the German and the English languages, that I have prepared the attached English translation of pages of a German Patent application in the German language with the title:

"Neue für das tal-Gen codierende Nukleotidsequenzen"

identified by the code number 990228 BT at the upper left of each page and corresponding to client/matter number of the law firm of

and that the attached English translation of this document is a true and correct translation of the document attached thereto to the best of my knowledge and belief.

I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001, and that such false statements may jeopardize the validity of this document.

By: _____ Dated: 31 March 2000

New nucleotide sequences which code for the tal gene

Field of the invention

The invention provides nucleotide sequences which code for the tal gene and a process for the fermentative preparation of amino acids, in particular L-lysine, L-threonine, L-isoleucine and L-tryptophan, using coryneform bacteria in which the tal gene is amplified.

Background of the invention

~~Prior art~~

Amino acids, in particular L-lysine, are used in human medicine and in the pharmaceuticals industry, but in particular in animal nutrition.

It is known that amino acids are prepared by fermentation by strains of coryneform bacteria, in particular Corynebacterium glutamicum. Because of their great importance, work is constantly being undertaken to improve the preparation processes. Improvements to the processes can relate to fermentation measures, such as e. g. stirring and supply of oxygen, or the composition of the nutrient media, such as e. g. the sugar concentration during the fermentation, or the working up to the product form by e. g. ion exchange chromatography, or the intrinsic output properties of the microorganism itself.

Methods of mutagenesis, selection and mutant selection are used to improve the output properties of these microorganisms. Strains which are resistant to antimetabolites, such as e. g. the lysine analogue S-(2-aminoethyl)-cysteine, or are auxotrophic for metabolites of regulatory importance and produce L-amino acids, such as e. g. L-lysine, are obtained in this manner.

Methods of the recombinant DNA technique have also been employed for some years for improving the strain of Corynebacterium strains which produce amino acids, by amplifying individual amino acid biosynthesis genes and investigating the effect on the amino acid production.

Review articles in this context are to be found, inter alia, in Kinoshita ("Glutamic Acid Bacteria", in: Biology of Industrial Microorganisms, Demain and Solomon (Eds.), Benjamin Cummings, London, UK, 1985, 115-142), Hilliger (BioTec 2, 40-44 (1991)), Eggeling (Amino Acids 6:261-272 (1994)), Jetten and Sinskey (Critical Reviews in Biotechnology 15, 73-103 (1995)) and Sahm et al. (Annuals of the New York Academy of Science 782, 25-39 (1996)).

The importance of the pentose phosphate cycle for the biosynthesis and production of amino acids, in particular L-lysine, by coryneform bacteria is the subject of numerous efforts among experts.

Thus Oishi and Aida (Agricultural and Biological Chemistry 29, 83-89 (1965)) report on the "hexose monophosphate shunt" of Brevibacterium ammoniagenes. Sugimoto and Shio (Agricultural and Bilogical [sic] Chemistry 51, 101-108 (1987)) report on the regulation of glucose 6-phosphate dehydrogenase in Brevibacterium flavum.

Object of the invention

The inventors had the object of providing new measures for improved fermentative preparation of amino acids, in particular L-lysine, L-threonine, L-isoleucine and L-tryptophan.

Summary ~~Description~~ of the invention

Amino acids, in particular L-lysine, are used in human medicine, in the pharmaceuticals industry and in particular in animal nutrition. There is therefore a general interest in providing new improved processes for the preparation of amino acids, in particular L-lysine.

When L-lysine or lysine are mentioned in the following, not only the base but also the salts, such as e. g. lysine monohydrochloride or lysine sulfate, are also meant by this.

The invention provides an isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence chosen from the group consisting of

a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4,

b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4,

c) polynucleotide which is complementary to the polynucleotides of a) or b) and

d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c).

The invention also provides the polynucleotide as claimed in claim 1, this preferably being a DNA which is capable of replication, comprising:

(i) a nucleotide sequence chosen from the group consisting of SEQ ID NO. 1 and SEQ ID NO. 3 or

(ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or

(iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally

(iv) sense mutations of neutral function in (i).

The invention also provides

a polynucleotide as claimed in claim 4, comprising one of the nucleotide sequences as shown in SEQ ID NO. 1 and SEQ ID NO. 3,

a polynucleotide as claimed in claim 5, which codes for a polypeptide which comprises the amino acid sequence as shown in SEQ ID NO. 2 and SEQ ID NO. 4,

a vector containing the polynucleotide as claimed in claim 1,

and coryneform bacteria, serving as the host cell, which contain the vector.

The invention also provides polynucleotides which substantially comprise a polynucleotide sequence, which are [sic] obtainable by screening by means of hybridization of a corresponding gene library, which comprises the complete gene with the polynucleotide sequence corresponding to SEQ ID NO. 1 or SEQ ID NO. 3, with a probe which comprises the sequence of the polynucleotide mentioned, according to

SEQ ID NO. 1 or SEQ ID NO. 3 or a fragment thereof, and isolation of the DNA sequence mentioned.

Polynucleotide sequences according to the invention are suitable as hybridization probes for RNA, cDNA and DNA, in order to isolate, in the full length, cDNA which code for transaldolase and to isolate those cDNA or genes which have a high similarity of sequence with that of the transaldolase gene.

Polynucleotide sequences according to the invention are furthermore suitable as primers for the preparation of DNA of genes which code for transaldolase by the polymerase chain reaction (PCR).

Such oligonucleotides which serve as probes or primers comprise at least 30, preferably at least 20, especially preferably at least 15 successive nucleotides. Oligonucleotides which have a length of at least 40 or 50 nucleotides are also suitable.

"Isolated" means separated out of its natural environment.

"Polynucleotide" in general relates to polyribonucleotides and polydeoxyribonucleotides, it being possible for these to be non-modified RNA or DNA or modified RNA or DNA.

"Polypeptides" is understood as meaning peptides or proteins which comprise two or more amino acids bonded via peptide bonds.

The polypeptides according to the invention include a polypeptide according to SEQ ID NO. 2 or SEQ ID NO. 4, in particular those with the biological activity of transaldolase, and also those which are identical to the extent of at least 70 % to the polypeptide according to SEQ ID NO. 2 or SEQ ID NO. 4, and preferably are identical to the extent of at least 80% and in particular to the extent of at least 90 % to 95 % to the polypeptide according to

SEQ ID NO. 2 or SEQ ID NO. 4, and have the activity mentioned.

The invention also provides a process for the fermentative preparation of amino acids, in particular L-lysine, L-threonine, L-isoleucine and L-tryptophan, using coryneform bacteria which in particular already produce an amino acid, and in which the nucleotide sequences which code for the tal gene are amplified, in particular over-expressed.

The term "amplification" in this connection describes the increase in the intracellular activity of one or more enzymes in a microorganism which are coded by the corresponding DNA, for example by increasing the number of copies of the gene or genes, using a potent promoter or using a gene which codes for a corresponding enzyme having a high activity, and optionally combining these measures.

Detailed Description of the Invention

The microorganisms which the present invention provides can prepare L-amino acids, in particular L-lysine, from glucose, sucrose, lactose, fructose, maltose, molasses, starch, cellulose or from glycerol and ethanol. They can be representatives of coryneform bacteria, in particular of the genus Corynebacterium. Of the genus Corynebacterium, there may be mentioned in particular the species Corynebacterium glutamicum, which is known among experts for its ability to produce L-amino acids.

Suitable strains of the genus Corynebacterium, in particular of the species Corynebacterium glutamicum, are, for example, the known wild-type strains

Corynebacterium glutamicum ATCC13032
Corynebacterium acetoglutamicum ATCC15806
Corynebacterium acetoacidophilum ATCC13870
Corynebacterium thermoaminogenes FERM BP-1539
Corynebacterium melassecola ATCC17965
Brevibacterium flavum ATCC14067

Brevibacterium lactofermentum ATCC13869 and
Brevibacterium divaricatum ATCC14020

and L-lysine-producing mutants or strains prepared therefrom, such as, for example

Corynebacterium glutamicum FERM-P 1709
Brevibacterium flavum FERM-P 1708
Brevibacterium lactofermentum FERM-P 1712
Corynebacterium glutamicum FERM-P 6463
Corynebacterium glutamicum FERM-P 6464 and
Corynebacterium glutamicum ATCC13032
Corynebacterium glutamicum DM58-1
Corynebacterium glutamicum DSM12866.

and L-threonine-producing mutants or strains prepared therefrom, such as, for example

Corynebacterium glutamicum ATCC21649
Brevibacterium flavum BB69
Brevibacterium flavum DSM5399
Brevibacterium lactofermentum FERM-BP 269
Brevibacterium lactofermentum TBB-10

and L-isoleucine-producing mutants or strains prepared therefrom, such as, for example

Corynebacterium glutamicum ATCC 14309
Corynebacterium glutamicum ATCC 14310
Corynebacterium glutamicum ATCC 14311
Corynebacterium glutamicum ATCC 15168
Corynebacterium ammoniagenes ATCC 6871

and L-tryptophan-producing mutants or strains prepared therefrom, such as, for example

Corynebacterium glutamicum ATCC21850 and
Corynebacterium glutamicum KY9218(pKW9901).

The inventors have succeeded in isolating the new tal gene of C. glutamicum which codes for transaldolase (EC 2.2.1.2).

To isolate the tal gene or also other genes of C. glutamicum, a gene library of this microorganism is first set up in E. coli. The setting up of gene libraries is described in generally known textbooks and handbooks. The textbook by Winnacker: Gene und Klone, Eine Einführung in die Gentechnologie [Genes and Clones, An Introduction to Genetic Engineering] (Verlag Chemie, Weinheim, Germany, 1990) or the handbook by Sambrook et al.: Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1989) may be mentioned as an example. A well-known gene library is that of the E. coli K-12 strain W3110 set up in λ vectors by Kohara et al. (Cell 50, 495-508 (1987)). Bathe et al. (Molecular and General Genetics, 252:255-265, 1996) describe a gene library of C. glutamicum ATCC13032, which was set up with the aid of the cosmid vector SuperCos I (Wahl et al., 1987, Proceedings of the National Academy of Sciences USA, 84:2160-2164) in the E. coli K-12 strain NM554 (Raleigh et al., 1988, Nucleic Acids Research 16:1563-1575). Börmann et al. (Molecular Microbiology 6(3), 317-326)) (1992)) in turn describe a gene library of C. glutamicum ATCC13032 using the cosmid pHC79 (Hohn and Collins, Gene 11, 291-298 (1980)). O'Donohue (The Cloning and Molecular Analysis of Four Common Aromatic Amino Acid Biosynthetic Genes from Corynebacterium glutamicum. Ph.D. Thesis, National University of Ireland, Galway, 1997) describes the cloning of C. glutamicum genes using the λ Zap expression system described by Short et al. (Nucleic Acids Research, 16: 7583). To prepare a gene library of C. glutamicum in E. coli it is also possible to use plasmids such as pBR322 (Bolivar, Life Sciences, 25, 807-818 (1979)) or pUC9 (Vieira et al., 1982, Gene, 19:259-268). Suitable hosts are, in particular, those E. coli strains which are restriction- and recombination-defective. An example of

these is the strain DH5αmcr, which has been described by Grant et al. (Proceedings of the National Academy of Sciences USA, 87 (1990) 4645-4649). The long DNA fragments cloned with the aid of cosmids can then in turn be subcloned and subsequently sequenced in the usual vectors which are suitable for sequencing, such as is described e. g. by Sanger et al. (Proceedings of the National Academy of Sciences of the United States of America, 74:5463-5467, 1977).

The DNA sequences obtained can then be investigated with known algorithms or sequence analysis programs, such as e. g. that of Staden (Nucleic Acids Research 14, 217-232(1986)),the GCG program of Butler (Methods of Biochemical Analysis 39, 74-97 (1998)) the FASTA algorithm of Pearson and Lipman (Proceedings of the National Academy of Sciences USA 85,2444-2448 (1988)) or the BLAST algorithm of Altschul et al. (Nature Genetics 6, 119-129 (1994)) and compared with the sequence entries which exist in databanks accessible to the public. Databanks for nucleotide sequences which are accessible to the public are, for example, that of the European Molecular Biologies Laboratories (EMBL, Heidelberg, Germany) of that of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA).

The invention provides the new DNA sequence from C.glutamicum which contains the DNA section which codes for the tal gene, shown as SEQ ID NO 1 and SEQ ID NO 3. The amino acid sequence of the corresponding protein has furthermore been derived from the present DNA sequence using the methods described above. The resulting amino acid sequence of the tal gene product is shown in SEQ ID NO 2 and SEQ ID NO 4.

A gene library produced in the manner described above can furthermore be investigated by hybridization with nucleotide probes of known sequence, such as, for example,

the zwf gene (JP-A-09224661). The cloned DNA of the clones which show a positive reaction in the hybridization is sequenced in turn to give on the one hand the known nucleotide sequence of the probe employed and on the other hand the adjacent new DNA sequences.

Coding DNA sequences which result from SEQ ID NO 3 by the degeneracy of the genetic code are also a constituent of the invention. In the same way, DNA sequences which hybridize with SEQ ID NO 3 or parts of ~~or [sic]~~ SEQ ID NO 3 are a constituent of the invention. Conservative amino acid exchanges, such as e. g. exchange of glycine for alanine or of aspartic acid for glutamic acid in proteins, are furthermore known among experts as "sense mutations" which do not lead to a fundamental change in the activity of the protein, i.e. are of neutral function. It is furthermore known that changes on the N and/or C terminus of a protein cannot substantially impair or can even stabilize the function thereof. Information in this context can be found by the expert, inter alia, in Ben-Bassat et al. (Journal of Bacteriology 169:751-757 (1987)), in O'Regan et al. (Gene 77:237-251 (1989)), in Sahin-Toth et al. (Protein Sciences 3:240-247 (1994)), in Hochuli et al. (Bio/Technology 6:1321-1325 (1988)) and in known textbooks of genetics and molecular biology. Amino acid sequences which result in a corresponding manner from SEQ ID NO 2 or SEQ ID NO 4 are also a constituent of the invention.

In the same way, DNA sequences which hybridize with ~~or~~ [s~~i~~c] SEQ ID NO 3 or parts of ~~or [sic]~~ SEQ ID NO 3 are a constituent of the invention. Finally, DNA sequences which are prepared by the polymerase chain reaction (PCR) using primers which result from SEQ ID NO 3 are a constituent of the invention. Such oligonucleotides typically have a length of at least 15 nucleotides.

Instructions for identifying DNA sequences by means of hybridization can be found by the expert, inter alia, in

the handbook "The DIG System Users Guide for Filter Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl et al. (International Journal of Systematic Bacteriology (1991) 41: 255-260).

Instructions for amplification of DNA sequences with the aid of the polymerase chain reaction (PCR) can be found by the expert, inter alia, in the handbook by Gait: ~~Oligonucleotide~~ Oligonucleotide [sic] synthesis: a practical approach (IRL Press, Oxford, UK, 1984) and in Newton and Graham: PCR (Spektrum Akademischer Verlag, Heidelberg, Germany, 1994).

The inventors have found that coryneform bacteria produce amino acids in an improved manner after over-expression of the tal gene.

To achieve an over-expression, the number of copies of the corresponding genes can be increased, or the promoter and regulation region or the ribosome binding site upstream of the structural gene can be mutated. Expression cassettes which are incorporated upstream of the structural gene act in the same way. By inducible promoters, it is additionally possible to increase the expression in the course of fermentative L-amino acid production. The expression is likewise improved by measures to prolong the life of the m-RNA. Furthermore, the enzyme activity is also increased by preventing the degradation of the enzyme protein. The genes or gene constructs can either be present in plasmids with a varying number of copies, or can be integrated and amplified in the chromosome. Alternatively, an over-expression of the genes in question can furthermore be achieved by changing the composition of the media and the culture procedure.

Instructions in this context can be found by the expert, inter alia, in Martin et al. (Bio/Technology 5, 137-146 (1987)), in Guerrero et al. (Gene 138, 35-41 (1994)), Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988)), in Eikmanns et al. (Gene 102, 93-98 (1991)), in European

Patent Specification EPS 0 472 869, in US Patent 4,601,893, in Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991), in Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)), in LaBarre et al. (Journal of Bacteriology 175, 1001-1007 (1993)), in Patent Application WO 96/15246, in Malumbres et al. (Gene 134, 15 - 24 (1993)), in Japanese Laid-Open Specification JP-A-10-229891, in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)), in Makrides (Microbiological Reviews 60:512-538 (1996)) and in known textbooks of genetics and molecular biology.

By way of example, the tal gene according to the invention was over-expressed with the aid of plasmids.

Suitable plasmids are those which are replicated in coryneform bacteria. Numerous known plasmid vectors, such as e. g. pZ1 (Menkel et al., Applied and Environmental Microbiology (1989) 64: 549-554), pEKEx1 (Eikmanns et al., Gene 102:93-98 (1991)) or pHS2-1 (Sonnen et al., Gene 107:69-74 (1991)) are based on the cryptic plasmids pHM1519, pBL1 or pGA1. Other plasmid vectors, such as e. g. those based on pCG4 (US-A 4,489,160), or pNG2 (Serwold-Davis et al., FEMS Microbiology Letters 66, 119-124 (1990)), or pAG1 (US-A 5,158,891), can be used in the same manner.

Plasmid vectors which are furthermore suitable are also those with the aid of which the process of gene amplification by integration into the chromosome can be used, as has been described, for example, by Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)) for duplication or amplification of the hom-thrB operon. In this method, the complete gene is cloned in a plasmid vector which can replicate in a host (typically E. coli), but not in C. glutamicum. Possible vectors are, for example, pSUP301 (Simon et al., Bio/Technology 1, 784-791 (1983)), pK18mob or pK19mob (Schäfer et al., Gene 145,

69-73 (1994)), pGEM-T (Promega ~~corporation~~ Corporation [sic], Madison, WI, USA), pCR2.1-TOPO (Shuman (1994). ~~[sic]~~ Journal of Biological Chemistry 269:32678-84; US-A 5,487,993), pCR® Blunt (Invitrogen, Groningen, Holland; Bernard et al., Journal of Molecular Biology, 234: 534-541 (1993)), pEM1 (Schrumpf et al, 1991, Journal of Bacteriology 173:4510-4516) or pBGS8 (Spratt et al.,1986, Gene 41: 337-342). The plasmid vector which contains the gene to be amplified is then transferred into the desired strain of C. glutamicum by conjugation or transformation. The method of conjugation is described, for example, by Schäfer et al. (Applied and Environmental Microbiology 60, 756-759 (1994)). Methods for transformation are described, for example, by Thierbach et al. (Applied Microbiology and Biotechnology 29, 356-362 (1988)), Dunican and Shivnan (Bio/Technology 7, 1067-1070 (1989)) and Tauch et al. (FEMS Microbiological Letters 123, 343-347 (1994)). After homologous recombination by means of a "cross over" event, the resulting strain contains at least two copies of the gene in question.

An example of a plasmid vector with the aid of which the process of amplification by integration can be carried out is pSUZ1, which is shown in figure 1. Plasmid pSUZ1 consists of the E. coli vector pBGS8 described by Spratt et al. (Gene 41: 337-342(1986)), into which the tal gene has been incorporated.

In addition, it may be advantageous for the production of amino acids to amplify or over-express one or more enzymes of the particular biosynthesis pathway, of glycolysis, of anaplerosis, of the pentose phosphate pathway or of amino acid export, in addition to the tal gene.

Thus, for example, for the preparation of L-amino acids, in particular L-lysine, one or more genes chosen from the group consisting of

- the dapA gene which codes for dihydrodipicolinate synthase (EP-B 0 197 335),
- the lysC gene which codes for a feed back resistant aspartate kinase (Kalinowski et al. (1990), Molecular and General Genetics 224: 317-324),
- the gap gene which codes for glycerolaldehyde 3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the pyc gene which codes for pyruvate carboxylase (DE-A-198 31 609),
- the mqo gene which codes for malate:quinone oxidoreductase (Molenaar et al., European Journal of Biochemistry 254, 395-403 (1998)),
- the tkt gene which codes for transketolase (accession number AB023377 of the databank of European Molecular Biologies Laboratories (EMBL, Heidelberg, Germany)),
- the gnd gene which codes for 6-phosphogluconate dehydrogenase (JP-A-9-224662),
- the zwf gene which codes for glucose 6-phosphate dehydrogenase (JP-A-9-224661),
- the lysE gene which codes for lysine export (DE-A-195 48 222),
- the zwa1 gene (DE 199 59 328.0; DSM 13115),
- the eno gene which codes for enolase (DE: 19947791.4),
- the devB gene,
- the opcA gene (DSM 13264)

can be amplified, preferably over-expressed, at the same time.

Thus, for example, for the preparation of L-threonine, one or more genes chosen from the group consisting of

- at the same time the hom gene which codes for homoserine dehydrogenase (Peoples et al., Molecular Microbiology 2, 63-72 (1988)) or the hom^{dr} allele which codes for a "feed back resistant" homoserine dehydrogenase (Archer et al., Gene 107, 53-59 (1991),
- the gap gene which codes for glycerolaldehyde 3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the pyc gene which codes for pyruvate carboxylase (DE-A-198 31 609),
- the mqo gene which codes for malate:quinone oxidoreductase (Molenaar et al., European Journal of Biochemistry 254, 395-403 (1998)),
- the tkt gene which codes for transketolase (accession number AB023377 of the databank of European Molecular Biologies Laboratories (EMBL, Heidelberg, Germany)),
- the gnd gene which codes for 6-phosphogluconate dehydrogenase (JP-A-9-224662),
- the zwf gene which codes for glucose 6-phosphate dehydrogenase (JP-A-9-224661),
- the thrE gene which codes for threonine export (DE 199 41 478.5; DSM 12840),
- the zwa1 gene (DE 199 59 328.0; DSM 13115),
- the eno gene which codes for enolase (DE: 19947791.4),

- the devB gene,

the opcA gene (DSM 13264)

can be amplified, preferably over-expressed, at the same time.. [sic]

It may furthermore be advantageous for the production of amino acids to attenuate

- the pck gene which codes for phosphoenol pyruvate carboxykinase (DE 199 50 409.1 DSM 13047) and/or
- the pgi gene which codes for glucose 6-phosphate isomerase (US 09/396,478, DSM 12969), or
- the poxB gene which codes for pyruvate oxidase (DE 199 51 975.7; DSM 13114), or
- the zwa2 gene (DE: 199 59 327.2; DSM 13113)

at the same time, in addition to the amplification of the tal gene.

In addition to over-expression of the tal gene it may furthermore be advantageous for the production of amino acids to eliminate undesirable side reactions (Nakayama: "Breeding of Amino Acid Producing Micro-organisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

The microorganisms prepared according to the invention can be cultured continuously or discontinuously in the batch process (batch culture) or in the fed batch (feed process) or repeated fed batch process (repetitive feed process) for the purpose of production of L-amino acids. A summary of known culture methods ~~are [sic]~~ described in the textbook by Chmiel (Bioprozeßtechnik 1. Einführung in die Bioverfahrenstechnik [Bioprocess Technology 1. Introduction to Bioprocess Technology (Gustav Fischer Verlag, Stuttgart,

1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen [Bioreactors and Peripheral Equipment] (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

The culture medium to be used must meet the requirements of the particular strains in a suitable manner. Descriptions of culture media for various microorganisms are contained in the handbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981). Sugars and carbohydrates, such as e. g. glucose, sucrose, lactose, fructose, maltose, molasses, starch and cellulose, oils and fats, such as e. g. soya oil, sunflower oil, groundnut oil and coconut fat, fatty acids, such as e. g. palmitic acid, stearic acid and linoleic acid, alcohols, such as e. g. glycerol and ethanol, and organic acids, such as e. g. acetic acid, can be used as the source of carbon. These substances can be used individually or as a mixture. Organic nitrogen-containing compounds, such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soya bean flour and urea, or inorganic compounds, such as ammonium sulphate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate, can be used as the source of nitrogen. The sources of nitrogen can be used individually or as a mixture. Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts can be used as the source of phosphorus. The culture medium must furthermore comprise salts of metals, such as e. g. magnesium sulfate or iron sulfate, which are necessary for growth. Finally, essential growth substances, such as amino acids and vitamins, can be employed in addition to the abovementioned substances. Suitable precursors can moreover be added to the culture medium. The starting substances mentioned can be added to the culture in the form of a single batch, or can be fed in during the culture in a suitable manner.

Basic compounds, such as sodium hydroxide, potassium hydroxide, ammonia or aqueous ammonia, or acid compounds, such as phosphoric acid or sulfuric acid, can be employed in a suitable manner to control the pH of the culture. Antifoams, such as e. g. fatty acid polyglycol esters, can be employed to control the development of foam. Suitable substances having a selective action, such as e. g. antibiotics, can be added to the medium to maintain the stability of plasmids. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, such as e. g. air, are introduced into the culture. The temperature of the culture is usually 20°C to 45°C, and preferably 25°C to 40°C. Culturing is continued until a maximum of L-amino acid has formed. This target is usually reached within 10 hours to 160 hours.

The analysis of L-amino acids can be carried out by anion exchange chromatography with subsequent ninhydrin derivatization, as described by Spackman et al. (Analytical Chemistry, 30, (1958), 1190).

The following microorganism has been deposited at the Deutsche Sammlung für Mikrorganismen und Zellkulturen (DSMZ = German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) in accordance with the Budapest Treaty:

- Escherichia coli JM109/pSUZ1 as DSM 13263.

SEQ ID NO 1 also contains the new devB gene. The process according to the invention is used for fermentative preparation of amino acids.

The following figures are attached:

Figure 1: Map of the plasmid pSUZ1

The abbreviations and designations used have the following meaning.

lacZ:	segments of lacZα gene fragment
kan r:	kanamycin resistance
tal:	transaldolase gene
ori:	origin of replication of plasmid pBGS8
BclI:	cleavage site of restriction enzyme BclI
EcoRI:	cleavage site of restriction enzyme EcoRI
HindIII:	cleavage site of restriction enzyme HindIII
PstI:	cleavage site of restriction enzyme PstI
SacI:	cleavage site of restriction enzyme SacI

a Detailed Description of the Invention
~~Examples~~

The following examples will further illustrate this invention. The molecular biology techniques, e.g. plasmid DNA isolation, restriction enzyme treatment, ligations, standard transformations of Escherichia coli etc. used are, (unless stated otherwise), described by Sambrook et al., (Molecular Cloning. A Laboratory Manual (1989) Cold Spring Harbour Laboratories, USA).

Example 1

Preparation of a genomic cosmid gene library from Corynebacterium glutamicum ATCC 13032

Chromosomal DNA from Corynebacterium glutamicum ATCC 13032 was isolated as described by Tauch et al. (1995, Plasmid 33:168-179) and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Code no. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany, Product Description SAP, Code no. 1758250). The DNA of the cosmid vector SuperCos1 (Wahl et al. (1987) Proceedings of the National Academy of Sciences USA 84:2160-2164), obtained from Stratagene (La Jolla, USA, Product Description SuperCos1 Cosmid Vector Kit, Code no. 251301) was cleaved with the restriction enzyme XbaI (Amersham Pharmacia, Freiburg, Germany, Product Description XbaI, Code no. 27-0948-02) and likewise dephosphorylated with shrimp alkaline phosphatase. The cosmid DNA was then cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Code no. 27-0868-04). The cosmid DNA treated in this manner was mixed with the treated ATCC13032 DNA and the batch was treated with T4 DNA ligase (Amersham Pharmacia, Freiburg, Germany, Product Description T4-DNA-Ligase, Code no.27-0870-04). Th

ligation mixture was then packed in phages with the aid of Gigapack II XL Packing Extracts (Stratagene, La Jolla, USA, Product Description Gigapack II XL Packing Extract, Code no. 200217). For infection of the E. coli strain NM554 (Raleigh et al. 1988, Nucleic Acid Research 16:1563-1575) the cells were taken up in 10 mM $MgSO_4$ and mixed with an aliquot of the phage suspension. The infection and titering of the cosmid library were carried out as described by Sambrook et al. (1989, Molecular Cloning: A laboratory Manual, Cold Spring Harbor), the cells being plated out on LB agar (Lennox, 1955, Virology, 1:190) with 100 µg/ml ampicillin. After incubation overnight at 37°C, recombinant individual clones were selected.

Example 2

Isolation and sequencing of the tal gene

The cosmid DNA of an individual colony was isolated with the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Product No. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Mannheim, Germany, Product Description SAP, Product No. 1758250). After separation by gel electrophoresis, the cosmid fragments in the size range of 1500 to 2000 bp were isolated with the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany). The DNA of the sequencing vector pZero-1, obtained from Invitrogen (Groningen, Holland, Product Description Zero Background Cloning Kit, Product No. K2500-01) was cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Product No. 27-0868-04). The

ligation of the cosmid fragments in the sequencing vector pZero-1 was carried out as described by Sambrook et al. (1989, Molecular Cloning: A laboratory Manual, Cold Spring Harbor), the DNA mixture being incubated overnight with T4 ligase (Pharmacia Biotech, Freiburg, Germany). This ligation mixture was then electroporated (Tauch et al. 1994, FEMS Microbiol Letters, 123:343-7) into the E. coli strain DH5αMCR (Grant, 1990, Proceedings of the National Academy of Sciences U.S.A., 87:4645-4649) and plated out on LB agar (Lennox, 1955, Virology, 1:190) with 50 µg/ml zeocin. The plasmid preparation of the recombinant clones was carried out with Biorobot 9600 (Product No. 900200, Qiagen, Hilden, Germany). The sequencing was carried out by the dideoxy chain-stopping method of Sanger et al. (1977, Proceedings of the National Academy of Sciences U.S.A., 74:5463-5467) with modifications according to Zimmermann et al. (1990, Nucleic Acids Research, 18:1067). The "RR dRhodamin Terminator Cycle Sequencing Kit" from PE Applied Biosystems(Product No. 403044, Weiterstadt, Germany) was used. The separation by gel electrophoresis and analysis of the sequencing reaction were carried out in a "Rotiphoresis NF Acrylamide/Bisacrylamide" Gel (29:1) (Product No. A124.1, Roth, Karlsruhe, Germany) with the "ABI Prism 377" sequencer from PE Applied Biosystems (Weiterstadt, Germany).

The raw sequence data obtained were then processed using the Staden program package (1986, Nucleic Acids Research, 14:217-231) version 97-0. The individual sequences of the pZero1 derivatives were assembled to a continuous contig. The computer-assisted coding region analysis [sic] were prepared with the XNIP program (Staden, 1986, Nucleic Acids Research, 14:217-231). Further analyses were carried out with the "BLAST search program" (Altschul et al., 1997, Nucleic Acids Research, 25:3389-3402), against the non-redundant databank of the "National Center for Biotechnology Information" (NCBI, Bethesda, MD, USA).

The nucleotide sequence obtained is shown in SEQ ID NO 1 and SEQ ID NO 3.

Example 3

Cloning of the tal gene

PCR was used to amplify DNA fragments containing the entire tal gene of C. glutamicum 13032 and flanking upstream and downstream regions. PCR reactions were carried out using oligonucleotide primers designed from the sequence as determined in examples 1 and 2. Genomic DNA was isolated from Corynebacterium glutamicum ATCC13032 according to Heery and Dunican (Applied and Environmental Microbiology 59: 791-799 (1993)) and used as template. The tal primers used were:

fwd. primer: 5' GGT ACA AAG GGT CTT AAG 3'C
rev. primer: 5' GAT TTC ATG TCG CCG TTA 3'

PCR Parameters were as follows:

35 cycles
95°C for 3 minutes
94°C for 1 minute
47°C for 1 minute
72°C for 45 seconds
2.0 mM MgCl2
approximately 150-200 ng DNA template.

The PCR product obtained was cloned into the commercially available pGEM-T vector purchased from Promega Corp. (pGEM-T Easy Vector System 1, cat. no. A1360, Promega UK, Southampton, UK) using strain E. coli JM109 (Yanisch-Perron et al., Gene, 33: 103-119 (1985)) as a host. The entire tal gene was subsequently isolated from the pGEM T-vector on an Eco RI fragment and cloned into the lacZα EcoRI site of the E. coli vector pBGS8 (Spratt et al., Gene 41(2-3): 337-342

(1986)). The restriction enzymes used were obtained from Boehringer Mannheim UK Ltd. (Bell Lane, Lewes East Sussex BN7 1LG, UK) and used according to manufacturer's instructions. E. coli JMI09 was then transformed with this ligation mixture and electrotransformants were selected on Luria agar supplemented with isopropyl-thiogalactopyranoside (IPTG), 5-bromo-4-chloro-3-indolyl-galactopyranoside (XGAL) and kanamycin at concentrations of 1mM, 0.02% and 50 mg/l respectively. Plates were incubated for twelve hours at 37°C. Plasmid DNA was isolated from one transformant, characterised by restriction enzyme analysis using Eco RI. This new construct was designated pSUZ 1.

SEQUENCE PROTOCOL

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Degussa-Hüls AG

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<400> 3

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Met Ser His Ile Asp Asp Leu Ala Gln Leu Gly Thr Ser Thr Trp Leu
 1               5                  10                  15
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```
gac gac ctc tcc cgc gag cgc att act tcc ggc aat ctc agc cag gtt   96
Asp Asp Leu Ser Arg Glu Arg Ile Thr Ser Gly Asn Leu Ser Gln Val
            20                  25                  30

att gag gaa aag tct gta gtc ggt gtc acc acc aac cca gct att ttc  144
Ile Glu Glu Lys Ser Val Val Gly Val Thr Thr Asn Pro Ala Ile Phe
        35                  40                  45

gca gca gca atg tcc aag ggc gat tcc tac gac gct cag atc gca gag  192
Ala Ala Ala Met Ser Lys Gly Asp Ser Tyr Asp Ala Gln Ile Ala Glu
    50                  55                  60

ctc aag gcc gct ggc gca tct gtt gac cag gct gtt tac gcc atg agc  240
Leu Lys Ala Ala Gly Ala Ser Val Asp Gln Ala Val Tyr Ala Met Ser
65                  70                  75                  80

atc gac gac gtt cgc aat gct tgt gat ctg ttc acc ggc atc ttc gag  288
Ile Asp Asp Val Arg Asn Ala Cys Asp Leu Phe Thr Gly Ile Phe Glu
                85                  90                  95

tcc tcc aac ggc tac gac ggc cgc gtg tcc atc gag gtt gac cca cgt  336
Ser Ser Asn Gly Tyr Asp Gly Arg Val Ser Ile Glu Val Asp Pro Arg
            100                 105                 110

atc tct gct gac cgc gac gca acc ctg gct cag gcc aag gag ctg tgg  384
Ile Ser Ala Asp Arg Asp Ala Thr Leu Ala Gln Ala Lys Glu Leu Trp
        115                 120                 125

gca aag gtt gat cgt cca aac gtc atg atc aag atc cct gca acc cca  432
Ala Lys Val Asp Arg Pro Asn Val Met Ile Lys Ile Pro Ala Thr Pro
    130                 135                 140

ggt tct ttg cca gca atc acc gac gct ttg gct gag ggc atc agc gtt  480
Gly Ser Leu Pro Ala Ile Thr Asp Ala Leu Ala Glu Gly Ile Ser Val
145                 150                 155                 160

aac gtc acc ttg atc ttc tcc gtt gct cgc tac cgc gag gtc atc gct  528
Asn Val Thr Leu Ile Phe Ser Val Ala Arg Tyr Arg Glu Val Ile Ala
                165                 170                 175

gcg ttc atc gag ggc atc aag cag gct gct gca aac ggc cac gac gtc  576
Ala Phe Ile Glu Gly Ile Lys Gln Ala Ala Ala Asn Gly His Asp Val
            180                 185                 190

tcc aag atc cac tct gtg gct tcc ttc ttc gtc tcc cgc gtc gac gtt  624
Ser Lys Ile His Ser Val Ala Ser Phe Phe Val Ser Arg Val Asp Val
        195                 200                 205

gag atc gac aag cgc ctc gag gca atc gga tcc gat gag gct ttg gct  672
Glu Ile Asp Lys Arg Leu Glu Ala Ile Gly Ser Asp Glu Ala Leu Ala
    210                 215                 220

ctg cgc ggc aag gca ggc gtt gcc aac gct cag cgc gct tac gct gtg  720
Leu Arg Gly Lys Ala Gly Val Ala Asn Ala Gln Arg Ala Tyr Ala Val
225                 230                 235                 240

tac aag gag ctt ttc gac gcc gcc gag ctg cct gaa ggt gcc aac act  768
Tyr Lys Glu Leu Phe Asp Ala Ala Glu Leu Pro Glu Gly Ala Asn Thr
                245                 250                 255

cag cgc cca ctg tgg gca tcc acc ggc gtg aag aac cct gcg tac gct  816
Gln Arg Pro Leu Trp Ala Ser Thr Gly Val Lys Asn Pro Ala Tyr Ala
            260                 265                 270
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```
gca act ctt tac gtt tcc gag ctg gct ggt cca aac acc gtc aac acc   864
Ala Thr Leu Tyr Val Ser Glu Leu Ala Gly Pro Asn Thr Val Asn Thr
        275                 280                 285

atg cca gaa ggc acc atc gac gcg gtt ctg gag cag ggc aac ctg cac   912
Met Pro Glu Gly Thr Ile Asp Ala Val Leu Glu Gln Gly Asn Leu His
    290                 295                 300

ggt gac acc ctg tcc aac tcc gcg gca gaa gct gac gct gtg ttc tcc   960
Gly Asp Thr Leu Ser Asn Ser Ala Ala Glu Ala Asp Ala Val Phe Ser
305                 310                 315                 320

cag ctt gag gct ctg ggc gtt gac ttg gca gat gtc ttc cag gtc ctg   1008
Gln Leu Glu Ala Leu Gly Val Asp Leu Ala Asp Val Phe Gln Val Leu
                325                 330                 335

gag acc gag ggt gtg gac aag ttc gtt gct tct tgg agc gaa ctg ctt   1056
Glu Thr Glu Gly Val Asp Lys Phe Val Ala Ser Trp Ser Glu Leu Leu
            340                 345                 350

gag tcc atg gaa gct cgc ctg aag tag                              1083
Glu Ser Met Glu Ala Arg Leu Lys
        355                 360
```

<210> 4
<211> 360
<212> PRT
<213> Corynebacterium glutamicum

<400> 4

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Met Ser His Ile Asp Asp Leu Ala Gln Leu Gly Thr Ser Thr Trp Leu
  1               5                  10                  15

Asp Asp Leu Ser Arg Glu Arg Ile Thr Ser Gly Asn Leu Ser Gln Val
            20                  25                  30

Ile Glu Glu Lys Ser Val Val Gly Val Thr Thr Asn Pro Ala Ile Phe
        35                  40                  45

Ala Ala Ala Met Ser Lys Gly Asp Ser Tyr Asp Ala Gln Ile Ala Glu
    50                  55                  60

Leu Lys Ala Ala Gly Ala Ser Val Asp Gln Ala Val Tyr Ala Met Ser
65                  70                  75                  80

Ile Asp Asp Val Arg Asn Ala Cys Asp Leu Phe Thr Gly Ile Phe Glu
                85                  90                  95

Ser Ser Asn Gly Tyr Asp Gly Arg Val Ser Ile Glu Val Asp Pro Arg
            100                 105                 110

Ile Ser Ala Asp Arg Asp Ala Thr Leu Ala Gln Ala Lys Glu Leu Trp
        115                 120                 125

Ala Lys Val Asp Arg Pro Asn Val Met Ile Lys Ile Pro Ala Thr Pro
    130                 135                 140

Gly Ser Leu Pro Ala Ile Thr Asp Ala Leu Ala Glu Gly Ile Ser Val
145                 150                 155                 160

Asn Val Thr Leu Ile Phe Ser Val Ala Arg Tyr Arg Glu Val Ile Ala
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                165                 170                 175

Ala Phe Ile Glu Gly Ile Lys Gln Ala Ala Ala Asn Gly His Asp Val
            180                 185                 190

Ser Lys Ile His Ser Val Ala Ser Phe Phe Val Ser Arg Val Asp Val
        195                 200                 205

Glu Ile Asp Lys Arg Leu Glu Ala Ile Gly Ser Asp Glu Ala Leu Ala
    210                 215                 220

Leu Arg Gly Lys Ala Gly Val Ala Asn Ala Gln Arg Ala Tyr Ala Val
225                 230                 235                 240

Tyr Lys Glu Leu Phe Asp Ala Ala Glu Leu Pro Glu Gly Ala Asn Thr
                245                 250                 255

Gln Arg Pro Leu Trp Ala Ser Thr Gly Val Lys Asn Pro Ala Tyr Ala
            260                 265                 270

Ala Thr Leu Tyr Val Ser Glu Leu Ala Gly Pro Asn Thr Val Asn Thr
        275                 280                 285

Met Pro Glu Gly Thr Ile Asp Ala Val Leu Glu Gln Gly Asn Leu His
    290                 295                 300

Gly Asp Thr Leu Ser Asn Ser Ala Ala Glu Ala Asp Ala Val Phe Ser
305                 310                 315                 320

Gln Leu Glu Ala Leu Gly Val Asp Leu Ala Asp Val Phe Gln Val Leu
                325                 330                 335

Glu Thr Glu Gly Val Asp Lys Phe Val Ala Ser Trp Ser Glu Leu Leu
            340                 345                 350

Glu Ser Met Glu Ala Arg Leu Lys
        355                 360
```

What is Claimed is:
~~Patent claims~~

1. An isolated polynucleotide from coryneform bacteria, ~~comprising~~ consisting of a polynucleotide sequence chosen from the group consisting of

a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4,

b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70 % to the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4

c) polynucleotide which is complementary to the polynucleotides of a) or b) and

d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequences of a), b) or c).

2. A polynucleotide as claimed in claim 1 wherein the polynucleotide is a preferably recombinant DNA which is capable of replication in coryneform bacteria and additionally contains at least one of the nucleotide sequences which codes for the genes tkt, zwf, opcA and devB.

3. A polynucleotide as claimed in claim 1, wherein the polynucleotide is an RNA.

4. A polynucleotide as claimed in claim 2, comprising one of the nucleotide ~~sequence [sic]~~ sequences as shown in SEQ ID NO. 3.

5. A polynucleotide as claimed in claim 2, which codes for a polypeptide which comprises the

amino acid sequence as shown in SEQ ID NO. 2 and SEQ ID NO. 4.

6. A DNA as claimed in claim 2 which is capable of replication, comprising

(i) a nucleotide sequence as shown in SEQ ID NO. 3, or

(ii) at least one sequence which corresponds to sequences (i) within the range of the degeneration of the genetic code, or

(iii) at least one sequence which hybridizes with the sequences complementary to sequences (i) or (ii), and optionally

(iv) sense mutations of neutral function in (i).

7. A coryneform bacterium serving as the host cell, which contains a vector which carries a polynucleotide as claimed in claim 1.

8. A process for the preparation of L-amino acids, which comprises
carrying out the following steps:

a) fermentation of the bacteria which produce the desired L-amino acid, in which at least the tal gene and optionally one or more of the genes tkt gene, zwt gene, devB gene or opcA gene are amplified at the same time,

b) concentration of the desired product in the medium or in the cells of the bacteria and

c) isolation of the desired L-amino acid.

9. A process as claimed in claim 8, wherein
bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally amplified are employed.

10. A process as claimed in claim 8, wherein bacteria in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly eliminated are employed.

11. A process as claimed in one ~~or more~~ of claims 8 to ~~12 [sic]~~, wherein coryneform bacteria which produce one of the amino acids from the group consisting of L-lysine, L-threonine, L-isoleucine or L-tryptophan are used.

12. A process for the fermentative preparation of L-amino acids, in particular lysine, as claimed in claim 8, wherein in the coryneform microorganisms which in particular already produce L-amino acids, one or more genes chosen from the group consisting of

12.1 the dapA gene which codes for dihydrodipicolinate synthase,

12.2 the lysC gene which codes for a feed back resistant aspartate kinase,

12.3 the gap gene which codes for glycerolaldehyde 3-phosphate dehydrogenase,

12.4 the pyc gene which codes for pyruvate carboxylase,

12.5 the mqo gene which codes for malate-quinone oxidoreductase,

12.6 the tkt gene which codes for transketolase,

12.7 the gnd gene which codes for 6-phosphogluconate dehydrogenase,

12.8 the zwf gene which codes for glucose 6-phosphate dehydrogenase,

12.9 the lysE gene which codes for lysine export,

12.10 the zwa1 gene,

12.11 the eno gene which codes for enolase,

12.12 the opcA gene

is or are amplified or over-expressed at the same time.

13. A process for the fermentative preparation of L-threonine as claimed in claim 8, wherein in coryneform microorganisms which in particular already produce L-threonine, one or more genes chosen from the group consisting of

13.1 at the same time the hom gene which codes for homoserine dehydrogenase or the hom^{dr} allele which codes for a "feed back resistant" homoserine dehydrogenase,

13.2 the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase,

13.3 the pyc gene which codes for pyruvate carboxylase,

13.4 the mqo gene which codes for malate:quinone oxidoreductase,

13.5 the tkt gene which codes for transketolase,

13.6 the gnd gene which codes for 6-phosphogluconate dehydrogenase,

13.7 the zwf gene which codes for glucose 6-phosphate dehydrogenase,

13.8 the thrE gene which codes for threonine export,

13.9 the zwal gene,

13.10 the eno gene which codes for enolase,

13.11 the opcA gene

is or are amplified, in particular over-expressed, at the same time.

14. A process as claimed in claim 10, wherein for the preparation of L-amino acids, in particular L-lysine, L-threonine, L-isoleucine or L-tryptophan, bacteria in which one or more genes chosen from the group consisting of, [sic]

14.1 the pck gene which codes for phosphoenol pyruvate carboxykinase

14.2 the pgi gene which codes for glucose 6-phosphate6 [sic] isomerase

14.3 the poxB gene which codes for pyruvate oxidase or

14.4 the zwa2 gene

is or are attenuated at the same time, are fermented.

15. A use of polynucleotide sequences as claimed in claim 1 as hybridization probes for isolation of the cDNA which codes for the tal gene product.

16. A use of polynucleotide sequences as claimed in claim 1 as hybridization probes for isolation of the cDNA or genes which have a high similarity with the sequence of the tal gene.

add a4

New nucleotide sequences which code for the tal gene

Abstract

The invention relates to an isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence chosen from the group consisting of

a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4,

b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70 % to the amino acid sequences of SEQ ID NO. 2 or SEQ ID NO. 4

c) polynucleotide which is complementary to the polynucleotides of a) or b) and

d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequences of a), b) or c)

and a process for the preparation of L-amino acids, which comprises carrying out the following steps:

a) fermentation of the desired L-amino acid-producing bacteria in which at least the tal gene is amplified,

b) concentration of the desired product in the medium or in the cells of the bacteria and

c) isolation of the L-amino acid.